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TECHNICAL MEMORANDUM 224

QUANTITATIVE DETERMINATION  
OF C<sup>14</sup> -PICLORAM DISTRIBUTION  
IN GIRDLED BEAN PLANTS

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NOVEMBER 1970

DEPARTMENT OF THE ARMY  
Fort Detrick  
Frederick, Maryland

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Plant Physiology Division  
PLANT SCIENCES LABORATORIES

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### ABSTRACT

Liquid scintillation techniques were used in conjunction with autoradiography and girdling to study  $C^{14}$ -picloram movement in Black Valentine beans following application to the primary leaf. Quantitative determinations were made of tissue samples from eight locations on each plant after 48 hours of treatment.

No  $C^{14}$ -picloram moved out of the primary leaf when its petiole was girdled, showing that the herbicide moves from the leaf via the symplast.

Girdling 2 cm above the primary leaf node enhanced movement of picloram into the apical portions of the plant, indicating that picloram is readily translocated in the xylem.

Plants were girdled 2 cm below the primary node to test the possible explanation that picloram moves basipetally from the primary leaf in the phloem with subsequent recycling to the xylem at the cotyledonary node. Acropetal translocation still occurred, which eliminated the absolute requirement for recycling. However, the fact that some recycling does occur was shown by the relatively small amounts of label moving upward in plants girdled 2 cm above the primary leaf node in combination with a girdle 2 cm below the cotyledonary node.

The requirement for phloem in the translocation of picloram was indicated by the drastic reduction of label in the apical portions of plants girdled both above and below the primary leaf node.

Migration from the phloem to the xylem is suggested as a major mode of picloram translocation in the bean plant. Picloram's rapid distribution throughout the plant in most species may be due to the fact that it readily utilizes both xylem and phloem in both foliar and root application. The translocation of picloram cannot be explained on the basis of movement solely within either the symplast or the apoplast, but instead involves the entire conduction system of the plant.

## I. INTRODUCTION

Picloram (4-amino-3,5,6-trichloropicolinic acid) has been reported as comparable to two other auxin-like herbicides, 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), with regard to its translocation characteristics<sup>1/</sup>. The translocation of 2,4-D and 2,4,5-T has been explained on the basis of mass flow in the phloem or via the transpiration stream from the roots to the apical region of the plant<sup>2/</sup>. Crafts reported the movement of 2,4-D as almost solely symplastic and further states that the movement of 2,4,5-T in leaves is also symplastic<sup>2/</sup>.

Earlier experiments with picloram<sup>3/</sup> indicated that it does not follow the same pattern of distribution as other auxin-like herbicides and that its translocation involves more than a solely symplastic movement. Davis, Merkle, and Bovey<sup>4/</sup> have reported that picloram is apparently transported in the xylem when applied to leaves of mesquite; however, Merkle and Davis<sup>5/</sup> indicate that xylem transport is not necessarily the explanation for picloram translocation in beans because moderate water stress had no significant effect on the translocation rate of picloram. Fisher, Bayer, and Weier<sup>6/</sup> reported that picloram is translocated from the leaf with the photosynthates in bean plants but also indicated that some movement is possible without photosynthesis.

In view of the seemingly conflicting reports, experiments were conducted to establish whether or not picloram is translocated in the same manner as 2,4-D and 2,4,5-T and to determine its probable mode of translocation.

## II. MATERIALS AND METHODS

Bean plants (*Phaseolus vulgaris* L. var. Black Valentine) were germinated in sand, transferred to aerated 0.5X Hoagland's nutrient solution, and grown in a controlled environmental chamber. The relative humidity of the growth chamber was 50±5% and the temperature 25±0.5 C. A 16-hour photoperiod of 1,460 ft-c illumination at plant-top level was provided by a mixture of fluorescent and incandescent bulbs.

Uniform plants in which the first trifoliate leaf had just opened (6 days old) were selected for use. Five series of plants were single- or double-girdled by three rotations in a jet of live steam in the following manner: in the middle of the petiole of the herbicide-treated primary leaf, 2 cm above the primary leaf node, 2 cm below the primary leaf node, and 2 cm above in combination with a girdle 2 cm below the primary leaf node, or 2 cm above the primary leaf node in combination with a girdle

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2 cm below the cotyledonary node. The girdled plants were supported by stakes for the duration of the experiment because only a thin strand of xylem, inadequate to hold the plant erect, remained after girdling.

One day after girdling, 50  $\mu$ l (0.05  $\mu$ c) of  $C^{14}$ -labeled picloram (carboxyl labeled) were applied to one primary leaf of each girdled plant. The labeled picloram in 95% ethanol was applied in five 10- $\mu$ l droplets to each of the five main leaf veins. After 48 hours the plants were harvested, quick-frozen in dry ice, and lyophilized until dry. The plants were pressed and placed on Kodak No-Screen X-ray film for autoradiographic evaluation<sup>2/</sup>.

Stem and leaf samples were removed from eight selected locations on the freeze-dried plants after the autoradiography of the whole plants was completed. These locations were: the terminal bud, 0.5 cm below the first trifoliolate leaf, 0.5 cm above the primary node, a disc of leaf tissue from the junction of the main veins of the untreated primary leaf, 0.5 cm below the primary node, 0.5 cm above the cotyledonary node, 0.5 cm below the cotyledonary node, and the stem-root transition zone. These tissue samples were digested as reported previously<sup>3/</sup> and counted in liquid scintillation to provide quantitative values of  $C^{14}$  distribution for comparison with autoradiographic images.

Additionally, a group of nongirdled and girdled bean plants were root-treated by the addition of unlabeled picloram to the nutrient solution to give a final concentration of 0.5  $\mu$ M. These plants were harvested 7 days after treatment and dry weights were obtained by oven drying at 95 C.

### III. RESULTS AND DISCUSSION

When plants were girdled 2 cm above the primary leaf node, autoradiographs showed strong images in the younger trifoliolate leaves as well as the axillary buds and stem. This distribution of  $C^{14}$ -picloram was substantiated in counts per milligram of tissue samples from these areas. More label moved to the apical portions of these plants than in the nongirdled  $C^{14}$ -picloram-treated control plant (Fig. 1 and 2).

Autoradiographs and counts from plants girdled below the primary leaf node also showed the presence of label in the trifoliolate leaflets (Fig. 3). There was, however, no indication of the presence of  $C^{14}$ -picloram basipetal to the girdle in these plants.

When plants were girdled 2 cm above and below the primary leaf node, there was only a slight indication of label acropetal to the upper girdle and no label basipetal to the lower girdle (Fig. 4).



FIGURE 1. Representative Autoradiograph of Nongirdled Picloram-Treated Plant. Specific activity of  $C^{14}$ -picloram expressed in counts/min per mg dry weight.



FIGURE 2. Representative Autoradiograph of Picloram-Treated Plant Girdled 2 cm Above Primary Node.

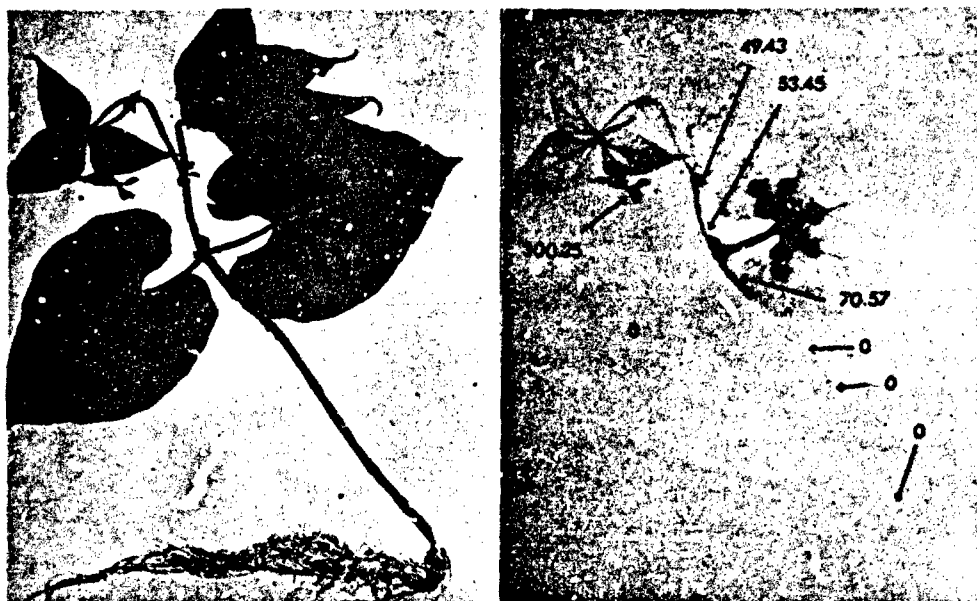


FIGURE 3. Representative Autoradiograph of Plant Girdled Below Primary Node

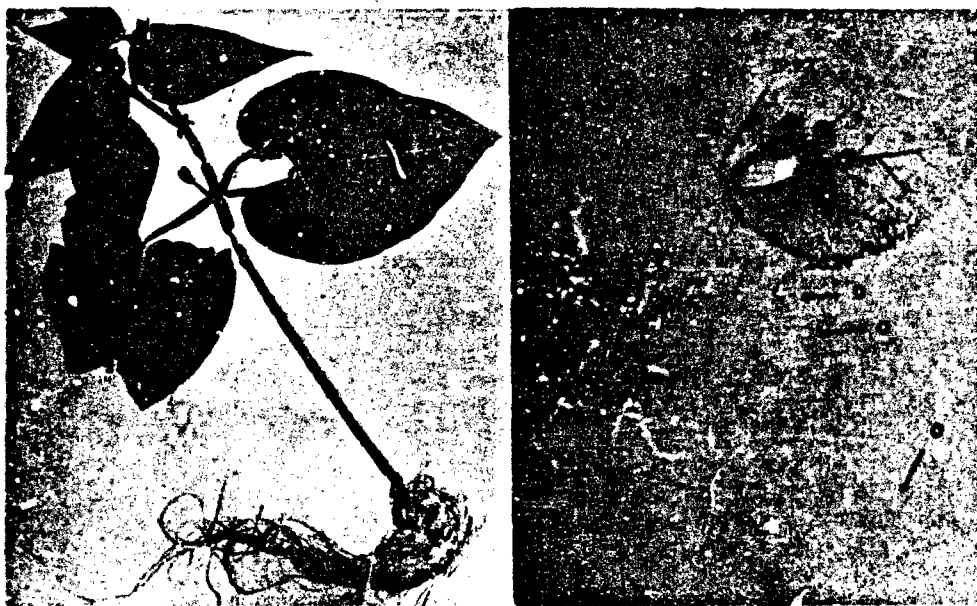


FIGURE 4. Representative Autoradiograph of Plant Girdled Above and Below the Primary Node.

Girdling the petiole of the treated leaf prevented all movement of  $C^{14}$ -picloram into the plant (Fig. 5). This indicates that picloram moves from the leaf via the symplast. If picloram follows the same distribution pattern as that of other auxin-like herbicides and is transported acropetally with the assimilates via the symplast, then girdling above the primary leaf node should prevent picloram from reaching the trifoliolate leaves and apical portions of the plant. However, the heavy labeling of the apical portions of plants girdled in this manner strongly implies movement of the picloram through the xylem. These plants also show the characteristic nastic response to picloram, which again indicates the presence of the herbicide in the apical portions of the plant. A possible mode of translocation for the picloram is migration to the xylem followed by upward movement in the transpiration stream. An additional possibility is movement of the picloram from the leaf basipetally in the phloem with subsequent recycling acropetally in the transpiration stream.

The latter possibility seems likely in light of the anatomy of the Black Valentine bean as reported by Douthett<sup>7/</sup>. According to Douthett, no vascular bundles pass upward from the primary leaves. She describes vascular connections with the opposite primary leaf and connections with the cotyledonary node but none passing acropetally from the primary node<sup>7/</sup>. If this anatomical arrangement of the vascular tissues of the bean is correct, then any substance moving from the primary leaves would first have to move basipetally as far as the cotyledonary node before it could move acropetally.

However, the appearance of  $C^{14}$  in the apical portions of those plants girdled between the primary leaf and the cotyledonary node indicates that unless migration has occurred between the primary leaf vascular bundles and those of the stem, there must exist some means of upward conduction from the primary leaves not reported by Douthett. Substances conducted only in the phloem (photosynthates) were also apparently moved upward directly from the primary leaves. This observation is supported by the fact that active growth of the trifoliolate leaves occurred in those plants girdled below the primary leaf node. The growth of the trifoliolates was inhibited in plants girdled above the node.

To further test the possibility of recycling at the cotyledonary node, a series of plants were girdled 2 cm above the primary node and 2 cm below the cotyledonary node. This combination presumably leaves a conduction pathway for substances to move downward from the primary leaves to the cotyledonary node, making possible recycling upward at this point. The appearance of relatively small amounts of label in the apical portions of plants girdled in this manner (Fig. 6) indicates that some recycling into the xylem does occur. No label is found in the terminal bud and only a minute amount of label is found in the upper stem of plants girdled both above and below the primary node (Fig. 4), a situation in which both recycling via the cotyledonary node and upward movement in the phloem are prohibited. This small amount of label must be the result of leakage from the phloem into the xylem. Such leakage of herbicides from the phloem to the xylem has been reported by other workers<sup>8-11/</sup>.



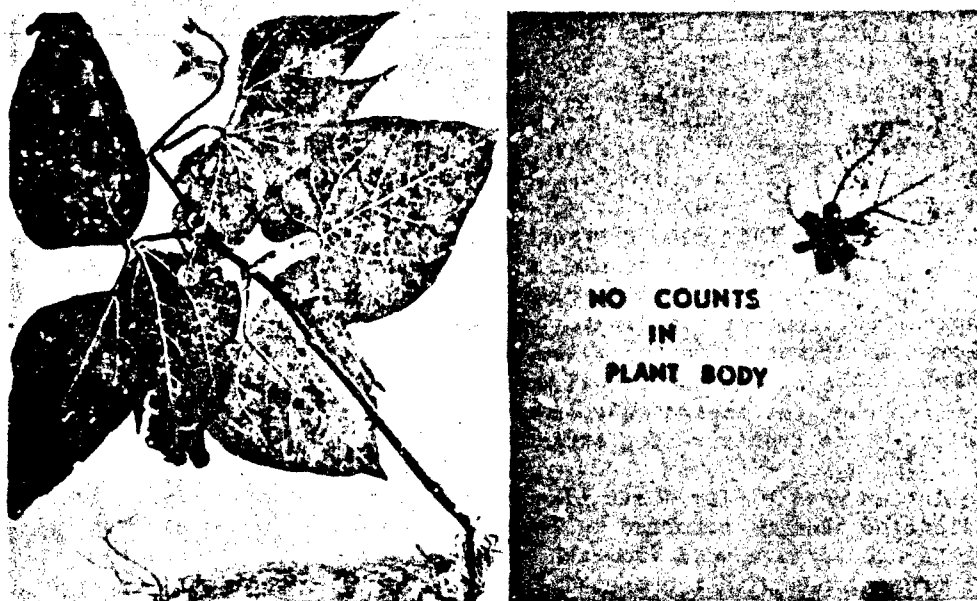


FIGURE 5. Representative Autoradiograph of Plant in Which the Petiole of the Picloram-Treated Primary Leaf has been Girdled.



FIGURE 6. Representative Autoradiograph of Plant Girdled Above the Primary Node and Below the Cotyledonary Node.

Although recycling does apparently occur in the plants girdled above the primary node and below the cotyledonary node, the quantity of label in the terminal bud is quite small in comparison with the amount reaching the apical portions in the case of plants girdled only above the primary node and only below the primary node. This indicates that, while some recycling does occur at the cotyledonary node, the major amount of picloram moving acropetally in girdled plants does so by some other means (probably via migration from the phloem to the xylem or via phloem tissue not noted by Douth in her anatomical study).

In other species there is some indication that herbicide exchange occurs between phloem and xylem. Microautoradiographs of stem tissues from green ash treated with  $C^{14}$ -2,4,5-T show heavy labeling of the vascular ray tissue. This clearly suggests that exchange between phloem and xylem occurs in this woody species\*. Crafts has stressed the importance of realizing that the conducting systems of plants, in particular the phloem, are in fact distribution systems and that movement of substances from the phloem is a natural function of that system<sup>12/</sup>. Eliasson<sup>9/</sup>, although in disagreement with Crafts on translocation modes of 2,4-D and 2,4,5-T, also reports that chlorophenoxy herbicides are easily transferred from phloem to xylem and calls for investigation of this phenomenon in other species.

Although the translocation of picloram in all of the above instances might be theoretically explained solely on the basis of migration and recycling, the necessity for the involvement of phloem is shown in plants girdled both above and below the primary leaf node. As mentioned above, there is only a small amount of label in the apical portions of these plants, nor is there any characteristic nastic response to picloram in the trifoliolate leaves. The indication that neither the symplast (phloem) nor the apoplast (xylem) is solely responsible for picloram translocation is also supported by observations of girdled plants to which picloram was root-applied via the nutrient solution. Dry-weight comparisons (Table 1) of the foliar portions of picloram-treated plants girdled and nongirdled 2 cm below the primary node indicate that growth inhibition by picloram was greater in those plants that had no interruption in the symplast, i.e., the ungirdled plants. This implies that picloram moves upward from the roots not only via the apoplast but also in the phloem.

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\* W. A. Wells, unpublished observations.

TABLE 1. COMPARISON OF PICLORAM-INDUCED GROWTH INHIBITION OF GIRDLED AND NON-GIRDLED BEAN PLANTS<sup>a</sup>/ GROWN IN A 0.5  $\mu$ M SOLUTION OF HERBICIDE AND 0.5X NUTRIENT SOLUTION

Picloram	Non-Girdled		Girdled		Decrease in Picloram Effect due to Girdling, %
	Dry Wt <sup>b</sup> /	Inhib, %	Dry Wt <sup>b</sup> /	Inhib, %	
0	2.287	0	1.676	0	-
50 $\mu$ M	0.756	67.0	0.696	58.5	12.7

- a. Black Valentine beans were 5 days old when girdled and 7 days old when placed in treatment solution.
- b. Values are means of eight to 10 replications and represent weight of shoots 7 days after treatment.

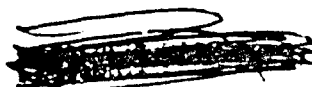
In summary, it is suggested that the mode of picloram translocation in the bean plant involves symplastic movement from the leaf with both acropetal and basipetal movement taking place at the primary leaf node. A single girdle either above or below the primary leaf node would hinder movement via the phloem in only one direction. In plants girdled below the primary leaf node, picloram as well as photosynthates apparently were readily translocated upward from the primary leaf despite the reported lack of vascular connections for acropetal movement from the primary leaves<sup>1</sup>. Apparently, recycling into the xylem at the level of the cotyledonary node does occur in plants girdled only above the primary node, but plants girdled above the primary node and below the cotyledonary node indicate that this mode may not account for the major movement of picloram to the foliar portions of the plant. Girdling above and below the primary leaf node greatly hindered movement of the picloram in either direction and suggests that migration to the xylem apparently does not occur within the immediate vicinity of the primary leaf node in double-girdled plants or again that unreported phloem bundles passing acropetally do exist. The possibilities also exist that the continuity of the symplast is too drastically disrupted by two girdles and is somehow essential for effective migration or that recycling also occurs at some point basipetal to the cotyledonary node.

Another point of interest is that only minute amounts of C<sup>14</sup>-picloram reach the primary leaf opposite the treated leaf in spite of the ample vascular connections between the two leaves reported by Douthett<sup>1</sup>.

The fact that picloram also moves basipetally in the phloem is indicated by the appearance of label in the lower stem portions of all plants not girdled at any point below the primary node.

Apparently, picloram is capable of moving independently of the photosynthates, because high amounts of label were found in the apical portions of plants girdled above the primary node while the growth of these portions was greatly inhibited.

These studies clearly show that picloram is readily transported in both the phloem and xylem, whether foliarly or root-applied. This phenomenon may explain the rapid distribution of picloram in most plant species. Resolution of conflicting reports regarding picloram translocation probably rests with realization that movement of the herbicide in the plant cannot be explained solely on the basis of transport within any one system, and that its movement in either of these systems may vary from one species to another. It is more plausible to consider that picloram translocation is not confined to either the symplast or apoplast but instead utilizes the entire conduction network of the plant.



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#### 14. Key Words

Picloram  
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Phloem  
Xylem  
Autoradiography  
Girdling  
Herbicides

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